Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2020, **12**, 4749

A new voltammetric platform for reliable determination of the sport performance-enhancing stimulant synephrine in dietary supplements using a boron-doped diamond electrode

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Received 24th July 2020
Accepted 21st August 2020

DOI: 10.1039/d0ay01425g

rsc.li/methods

For the first time, we propose a new modification-free voltammetric platform for simple, fast and reliable quantification of the sport performance-enhancing stimulant synephrine (SYN) based on the usage of a commercial screen-printed boron-doped diamond (BDD) electrochemical sensor. Applying cyclic voltammetry, SYN exhibited one well-shaped, irreversible and diffusion-driven oxidation peak at a peak potential of +1.45 V (*vs.* the silver pseudoreference electrode) in the presence of 2 M $HClO_4$. Under the selected experimental conditions, the following analytical parameters for determination of SYN were obtained: linear concentration ranges from 19.6 up to 1000 μM and from 9.9 up to 1000 μM for differential pulse (DPV) and square-wave voltammetry (SWV), respectively, detection limits at micromolar concentration levels and intra-day repeatability with a relative standard deviation below 4% for both pulse techniques. The interference study revealed fair selectivity when considering the target dietary supplements. The feasibility of the developed voltammetric platform was verified in the analysis of commercially available dietary supplements and reasonable recovery values were achieved by DPV and SWV, respectively. In addition, the method can be performed directly without higher consumption of chemicals, waste generation, complex sample extraction and higher instrumentation cost. The advanced BDD electrochemical sensor has appeared to be a suitable competitor for efficient applications in food quality control analysis.

1. Introduction

p-Synephrine (SYN, IUPAC name: 4-[1-hydroxy-2-(methylamino) ethyl]phenol) is an alkaloid that occurs naturally in citrus fruits, particularly in bitter orange (*Citrus Aurantium*). It is also known as a substance which is structurally similar to adrenaline and ephedrine. Since the ban on ephedrine in dietary supplements by the U.S. Federal Drug Administration in 2004 due to its adverse effects on human health, SYN is increasingly being used as its permissible substitute (called also "fat burners").[1] The main reason lies in the accompanying effects, especially targeted at weight loss, reduction of fat accumulation and performance enhancement for athletes (energizing and muscle-enhancing agents) *via* stimulation of lipolysis or by inhibition of lipogenesis.[2] As evidenced in many EU countries, there is no legislation which may limit the content of SYN and other alkaloids in dietary supplements. Therefore, needful consideration should be given to improve the legislation and surveillance systems in order to provide consumers with nutritional security and protection against adulteration.[3] On the other hand, a maximum permitted amount of SYN may be desirable as very similar adverse effects to ephedrine, such as cardiac arrhythmias, flushing, nausea, insomnia and pulmonary edema, cannot be fully excluded.[4,5] As long as insufficient information on the safety of SYN is available, no safe dose for humans may currently be inferred.

Considering the medical application, it is generally known that SYN can increase blood pressure. This aspect is relevant because people (who are usually overweight) taking SYN-containing preparations are particularly at risk from cardiovascular diseases.[6] Besides, SYN may also interact with other drugs affecting their efficacy.[7] Medicinal products containing SYN are registered in several European countries. In addition, they are not considered to be prohibited substances by the World Anti-Doping Agency (WADA), although SYN itself has been more closely included in the monitoring program.[8] The oral absorption of SYN in humans is rapid and complete, with maximum plasma concentrations reached after 1 to 2 h. Following oral administration of 6 mg of SYN, approximately

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80% is excreted in the urine within 24 h and only 2.5% of the dose is excreted as SYN.[9] Despite a legitimate concern from the doctors, pharmacologists and scientists in this field, SYN-containing products do not appear to be as dangerous as ephedrine and remain legally available today. However, it should be emphasized that a maximum limit for SYN (30 mg day^{-1})[10] should be combined with labeling requirements and possibly with requirements for caffeine as people should take a higher dose of SYN than the recommended one immediately before their workout. Taking into account the potential health risk of SYN for people performing sports, the development of simple, rapid, accessible, low-cost and green analytical methods and procedures for quality control of dietary products as well as for clinical diagnosis is of first-rate importance. Special care has to be taken to assess whether the determined results of SYN amounts in supplements are reliable and similar to those as indicated by the manufacturer on the labels.

The latest literature survey has revealed numerous studies describing various instrumental analytical methods for detection and quantification of SYN in several matrices. Liquid chromatography may be considered one of the most dominant analytical methods for SYN sensing, in conjunction with different spectral detection techniques such as UV (DAD),[11] fluorescence,[12] chemiluminescence,[13] MS[14] and MS/MS.[15] Among others, gas chromatography,[16] capillary electrophoresis[17] and spectroscopy[18] are also preferred methods for quantification of SYN. In spite of the significant sensitivity and selectivity of these methods (especially chromatographic ones), most of them possess several drawbacks that could make them unsuitable for on-site routine analysis. These comprise expensive instrumentation, laborious analysis and necessity of using toxic organic solvents, which in combination with long-term sample preparation and the requirement of well-trained operators, drastically increased the time, environmental friendliness and overall cost of the analysis process. Accordingly, simple, fast, efficient and greener analytical methods for quantification of SYN in various matrices are still indispensable.

The recent and remarkable aspects in analytical chemistry in the last decade concern the growing use of modern electroanalytical methods, particularly for reliable analysis of drug formulations and dietary supplements. These methods have been gaining prominence and are characterized as simple, rapid, low-cost, and user-convenient with a low matrix effect and the possibility of miniaturization of the experimental set-up and using portable electrochemical devices.[19] This can also be achieved with colored materials or with dispersed solid particles; thus this type of analysis could be very convenient for routine analysis. In addition, an investigation of drug redox behavior may significantly open up their further therapeutic potential.[20] With regard to the validation parameters, modern electroanalytical methods are also considered to be sufficiently sensitive, precise and accurate, which constantly brings them to the forefront in the field of instrumental analytical methods.[21] The intense advancement of these methods is principally due to the development and successful application of state-of-the-art and advanced carbon materials and structures (graphene, quantum dots, nanotubes/nanoparticles, carbon black, boron-doped diamonds, *etc.*) with distinguished performance for the charge transfer process.

In spite of the presence of an oxidizing phenolic hydroxyl group in the structure of the SYN molecule, however, until now there have been only a few reports on the electrochemical study and/or determination of this compound in the scientific literature. Furthermore, the data from the literature indicate that significant attention has been recently paid to the utilization of chemically modified electrodes owing to the excellent physical and chemical properties, activity and large active surface area of a particular modifier enhancing the sensitivity and selectivity. In this respect, Liu *et al.* first developed a sensitive and fast sensor based on a glassy carbon electrode (GCE) modified with a multiwalled-carbon nanotube (MWCNT)/Nafion film for the quantification of SYN in Chinese traditional herbal drugs.[22] The linear concentration range from 10 nM to 10 μM with a low detection limit (LOD) of 8 nM was achieved by linear sweep voltammetry. Recently, Gao *et al.* designed and introduced a rapid and simple method based on an MXene/MWCNT/GCE sensor for the electrochemical determination of SYN.[23] Due to the excellent electrocatalytic activity and high conductivity of MWCNTs, the large surface area of MXene and the synergistic effect between MXenes and MWCNTs, the sensor demonstrated a wide concentration range of 0.5–70 μM and a low LOD (0.17 μM) towards SYN. The sensor was also applied for the assessment of the SYN amount in *Fructus Aurantii* samples with adequate results. The "gate effect" mechanism for conductive molecularly imprinted polymer (MIP) film coated electrodes for indirect electrochemical determination of SYN was investigated by Lach *et al.*[24] For this purpose, the SYN imprinted polythiophene-based film was deposited *via* electropolymerization under potentiodynamic conditions. The authors demonstrated that the decrease of the differential pulse voltammetric peak current for the $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ redox probe with the increase of the SYN concentration did not originate from swelling or shrinking of the MIP film. Instead, this decrease was caused by the decline of the electrochemical reversibility of the redox probe. The achieved LOD for SYN was 12 nM. Undoubtedly, the above mentioned electrochemical sensors achieve very high sensitivity and low LODs for SYN determination when modifying their origin electrode surface. However, it should be taken into account that the preparation of chemically modified electrodes is often time-consuming and more expensive with messy modification protocols including the complex process of incorporation of a modifier onto the electrode surface and frequently comprises synthesis of polymeric matrices and composites. All these aspects may result in substantially higher background current and unrepeatable results, mostly as a consequence of the non-reproducibility of the preparation of the particular chemically modified electrode.[25]

The boron-doped diamond (BDD) has been broadly used as a progressive electrode material for miscellaneous electrochemical purposes, in particular as a powerful sensor in drug, food and environmental analysis[26–28] as well as an efficient tool in electro-oxidation processes for wastewater treatment.[29,30] Because of the favorable attributes of this advanced material

such as very wide potential range, minor or negligible propensity to adsorption, low background currents, chemical and mechanical stability, biocompatibility and commercial availability,[31–33] the BDD electrode has been popularly used as an eligible alternative to conventional carbon-based (glassy carbon, carbon paste and graphite) and chemically modified electrodes. Over the last few years, a lot of effort was made by our working group using different types of BDD electrodes to determine various structurally interesting compounds in the field of clinical,[34] pharmaceutical,[35] food[36] and environmental[37] studies.

In accordance with the above stated knowledge, the main objective of this work was to develop a novel, simple, fast and reliable voltammetric platform for SYN quantification based on application of commercial screen-printed BDD electrodes in conjunction with differential pulse and square-wave voltammetry. The viability of the proposed methodology was endorsed by the analysis of commercially available dietary supplements with the declared amount of SYN. It is also worth noting that the proposed paper signifies the very first modification-free approach for the study of the electrochemical behavior and determination of SYN using the BDD electrode.

2. Experimental

2.1 Reagents

The reference material of SYN (purity > 98%) was obtained from Sigma-Aldrich and used without further purification. The deionized water with a resistivity not less than 18.2 MΩ cm was used for the preparation of various supporting electrolytes such as Britton–Robinson (BR) buffers and various strong inorganic acids (HCl, H_2SO_4, HNO_3, $HClO_4$, p.a., all from Lachema, Czech Republic) as well as for the preparation of all stock and working solutions of SYN and interfering agents. BR buffers were prepared by mixing acetic acid, phosphoric acid and boric acid (all components at a 40 mM concentration, p.a., Lachema, Czech Republic) with subsequent adjustment to the required pH by the addition of a certain volume of 0.2 M NaOH. The stock solution of SYN (10 mM) was prepared by dissolution of 167.2 mg reference material of SYN in a small aliquot of water which was subsequently filled up to the mark in a 100 mL volumetric flask. This stock solution was constantly stored in a refrigerator (when not used) without any consistency changes for a few weeks. Working solutions of lower SYN concentrations were always freshly prepared on the particular day of the experiment. All stock solutions were stored in a refrigerator at 4 °C. The experiments were carried out at surrounding laboratory temperature.

2.2 Instrumentation

All voltammetric measurements were guided with an Autolab PGSTAT 101 (Metrohm Autolab B.V., The Netherlands) in connection with electrochemical software NOVA (version 1.9). The complete three-electrode system was bought from DropSens, Asturias, Spain at a miniature scale (commercially marketed as CBDD110). It was placed on a ceramic substrate consisting of the thick-film bare BDD working electrode (boron doping level of 2500 ppm, diameter of 3.6 mm), silver pseudoreference electrode and screen-printed carbon auxiliary electrode.[36] The pH values were assessed using a pHenomenal pH 1100L meter (VWR, Slovakia) with a combined glass–reference electrode.

2.3 Voltammetric procedures

The initial electrochemical behavior study of SYN for the selection of suitable experimental conditions including choice of supporting electrolyte and effect of scan rate was performed using cyclic voltammetry (CV). The pulse voltammetric techniques such as differential pulse voltammetry (DPV) and square-wave voltammetry (SWV) were employed for the quantification of SYN. They were used just after selection of the suitable values of instrumental parameters involving modulation amplitude, modulation time and frequency. After finding the appropriate experimental conditions, the calibration lines were obtained by a successive addition of aliquots of 0.1 mM SYN working solution into the electrochemical cell already containing the supporting electrolyte. Thus *in situ* prepared calibration solutions were each analyzed in triplicate. As SYN is an oxidizable compound, no deoxygenation needed to be completed prior to analysis. In regard to working electrode cleaning, before starting new voltammetric analysis the BDD electrode surface was always thoroughly washed with deionized water without any additional mechanical polishing procedure and/or electrochemical (hydrogen, oxygen) pretreatment. OriginPro® 8.0 (OriginLab Corporation, USA) was used for data evaluation using the linear least-squares regression. The LOD was calculated as three times the standard deviation of the current response for the blank solution divided by the slope of the calibration curve.

2.4 Sample preparation

Four different caffeine-free SYN-containing products (supplied as powders in tablet form) were ordered and bought from various commercial companies focusing on selling nutritional supplements for athletes. The sample for analysis was prepared as follows: five tablets of a particular brand of supplement were thoroughly crushed in a mortar to a white powder. Subsequently, 0.1000 g of a powder was weighed and dissolved in 10 mL of methanol and then filtered. The resulting filtrate was quantitatively transferred to a 100 mL volumetric flask and made up to the mark with deionized water to obtain the stock sample solution. For the determination of SYN in the corresponding dietary supplement, 1 mL of the stock sample solution was always pipetted into the electrochemical cell already containing 10 mL of supporting electrolyte. The concentration of SYN in the particular sample was determined by the multiple standard addition method. This method consisted of gradually adding certain volumes from 0.8 to 3 mL of 1 mM SYN working solution to the electrochemical cell, depending on the declared amount of SYN in the particular product. The solution was thoroughly mixed after each standard addition and analyzed

using the DPV and SWV techniques ($n = 3$) with the appropriate values of operating parameters.

3. Results and discussion

3.1 Selection of the supporting electrolyte and effect of pH

In the initial step, attention was paid to the investigation of the electrochemical activity of 1 mM SYN on the BDD electrode using CV with a scan rate of 100 mV s^{-1}. The suitable experimental conditions were considered to obtain a favorable current response for the analyte in terms of sensitivity, selectivity and repeatability. One of the crucial experimental parameters to be studied was the pH of the supporting electrolyte used. For this purpose, the pH effect of the BR buffer on the current response of SYN was first monitored. Fig. 1 displays the total voltammetric profile for the electrochemical activity of 1 mM SYN in the potential window from 0 to +2 V on the BDD electrode in the presence of BR buffers in the pH range of 2–12. At first glance it is obvious that the studied alkaloid exhibits considerable electrochemical activity on this progressive carbon-based electrode material, which depending on the pH, leads to the presence of one or two oxidation peaks. In an acidic environment (pH 2–6), only one oxidation peak of SYN was observed in the potential region of about +1.5 V, while in a neutral medium the onset of the second emerging signal at a lower peak potential of approximately +0.8 V was already beginning to be visible in the CV curves. In an alkaline environment (pH 8–12), both native and new emerging oxidation peaks were observed in the voltammetric profile of SYN on the BDD electrode. This fact partially corresponds to the electrochemical behavior of SYN investigated on MWCNTs/Nafion/GCE.[22] However, in that study, Liu *et al.* recorded only one oxidation peak of SYN at about +1 V, but they explored the analyte behavior only in acidic media (pH range of 1.81–6.86) and at the same time in a limited potential range (from +0.6 to +1.3 V). Similarly, Gao *et al.* recorded only one oxidation peak of SYN on MXene/MWCNTs/GCE when examining the pH effect on analyte current response, albeit in a restricted pH range (5–7) and potential range (from +0.2 V to +1 V).[23] Therefore, the results presented herein may significantly contribute to completely clarifying the electrochemical behavior of SYN in the whole pH range on bare (unmodified) working electrode as there is evidently a lack of information in the scientific literature concerning the electro-activity of SYN.

As is evident from Fig. 1, with the increasing pH of BR buffer, the peak current magnitude (I_p) of 1 mM SYN decreased continuously from pH 3 (red thick line) to pH 12, while at the same time the background current gradually increased. Comparing the I_p values within the pH range of 2–6 it could be stated that the highest magnitude (10.9 μA) with the favorable peak performance was recorded when BR buffer with pH 3 was used. For this buffer, the CV curve with the absence of the analyte indicating a low background current on the BDD electrode is also depicted in Fig. 1. Moreover, the results also revealed that the employment of BR buffers from pH 8 to 12 was not convenient for the electrochemical sensing of SYN on the BDD electrode. This is due to a decline of I_p values for the native SYN signal at approximately +1.5 V caused by an overlap with the potential of the discharged electrolytic solution. This led to the fact that at pH 12 this peak was practically no longer observed in the CV curves. Nevertheless, the second emerging peak of SYN at +0.8 V with low intensity was observed in BR buffers with pH higher than 6. For instance, its I_p at pH 12 was 2–3-fold smaller compared to that for the native SYN peak at about +1.5 V recorded at pH 3. Finally, the more suitable and repeatable CV curves were obtained in the pH range of 2–6 (Fig. 2) affirming the fact that the acidic environment could be agreeable for the electrochemical activity of SYN on the BDD electrode. In addition, in more acidic environments, the observed background current appeared to be low at higher potentials (*e.g.*, 1.5 μA at a potential of +1.5 V in BR buffer with pH 3), probably due to the water oxidation which produces $^{\bullet}OH$ radicals as products, thus enhancing the electrode reaction of the analyte.[36] This phenomenon demonstrates the advantages

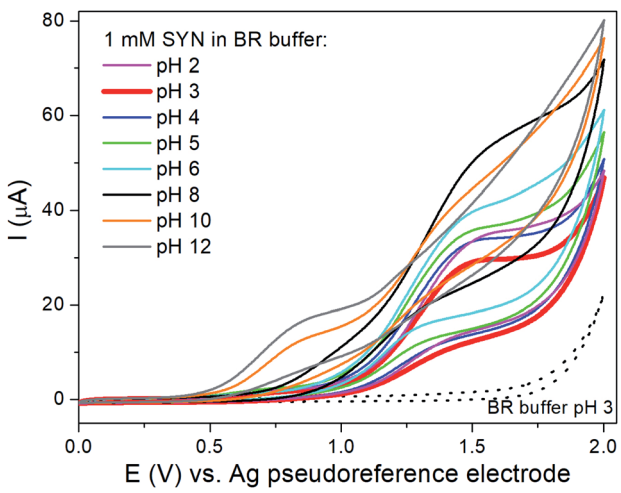


Fig. 1 CV profiles of 1 mM SYN recorded in the whole pH range (2–12) of the BR buffer on the BDD electrode with a scan rate of 100 mV s^{-1}.

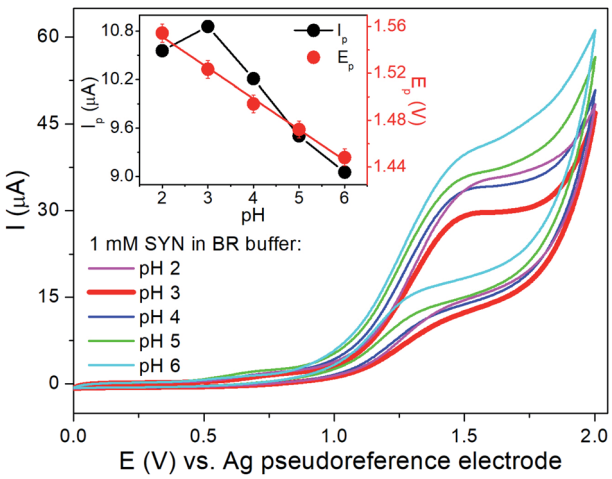


Fig. 2 CV profiles of 1 mM SYN at selected pH values (2–6) of BR buffer on the BDD electrode with a scan rate of 100 mV s^{-1}. The effect of pH on the peak potential (E_p) and the peak current (I_p) of SYN is shown in the inset dependences inside the figure.

and legitimacy of using the BDD electrode as an efficient electrochemical sensor for determination of highly oxidizing analytes.[25,32,36,37] To complete the characterization of the electrochemical behavior of SYN, on reversing the voltammetric scan no cathodic peak of the analyte appeared regardless of the pH values, indicating that the oxidation mechanism of SYN on the BDD electrode was irreversible.

Furthermore, if we take a closer look into the electrochemical behavior study for the pH range of 2–6 in Fig. 2, there is a slight shift of the peak potential of 1 mM SYN towards less positive values (from +1.55 to +1.44 V) clearly proving the protonation–deprotonation properties of SYN within the electrode reaction. Fig. 2 also points out the dependence of the peak potential (E_p) and peak current (I_p) of SYN evaluated from CV profiles on the pH of BR buffer. A plot of E_p against pH showed a linear dependence which can be expressed according to following equation (eqn (1)):

$$E_p\ (\mathrm{V}) = 1.604(\pm 0.005) - 0.028(\pm 0.001) \times \mathrm{pH},\ (R^2 = 0.9929) \quad (1)$$

Generally, a Nernstian slope of 0.059 V per pH at 25 °C stands for an electrochemical mechanism regarding equal number of electrons and protons.[38] Taking into account the slope value of 0.028 V per pH in eqn (1), the electrochemical oxidation of SYN on the BDD electrode is believed to occur *via* two electron and one proton transfer. A deeper postulate interpretation is given separately in Section 3.3.

Bearing in the mind the fact that acidic media could be considered a suitable environment for the electrochemical oxidation of SYN, we scrutinized several strong inorganic acids as possible supporting electrolytes including HCl, HNO_3, $HClO_4$ and H_2SO_4, all in the concentration range from 0.1 to 2 M. The effect of the presence of these acids on the current response of 1 mM SYN using CV on the BDD electrode is shown in Fig. 3. Obviously, when HCl was used, the oval-shaped oxidation peaks of SYN were recorded at high potentials (approximately at +1.7 V), but with low intensity and high background current in the case of all the studied acid concentrations. Consequently, this environment was evaluated to not be convenient due to the weaker electrochemical activity of SYN on the working electrode. Concerning HNO_3, the voltammetric profile of SYN was defined by the presence of sharper oxidation peaks which were observed at lower potentials (just below +1.5 V). Herein, the most suitable performance of the current response of the analyte was achieved in 1 M HNO_3. However, utilizing $HClO_4$ as the supporting electrolyte, well-shaped CV curves were recorded for SYN with E_p ranging from +1.4 to +1.5 V and the most appropriate current response at the 2 M concentration level. It should also be underlined that these voltammograms were almost identical in shape and intensity (20 μA) to those recorded in H_2SO_4. Nevertheless, a more detailed study and evaluation of the CV curves recorded in 2 M H_2SO_4 revealed that the particular oxidation peaks of SYN in this medium were less repeatable, leading to a decrease of the current response in subsequent CV scans (the results are not shown). On the other hand, in the case of 2 M $HClO_4$, the SYN signal was more stable during repeated CV scans. Accordingly, this electrolyte was chosen for further measurements. In addition, for instance, when comparing the current response of SYN in BR buffer with pH 3 in the initial measurements with the value observed in 2 M $HClO_4$, it was found that the studied analyte in the presence of 2 M $HClO_4$ gave a two times higher peak current than in BR buffer with pH 3. For comparison, in previous work dealing with the electroanalytical determination of SYN, BR buffer with pH 2.4 (ref. 22) and PBS with pH 7 (ref. 23) were applied as supporting electrolytes.

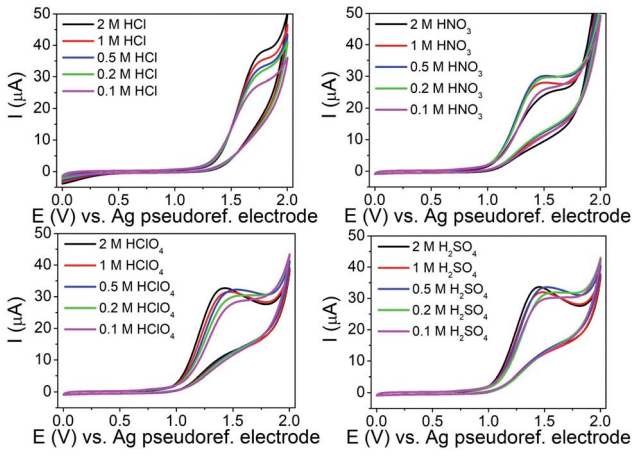


Fig. 3 CV profiles of 1 mM SYN in the presence of various concentrations (0.1–2 M) of inorganic acids: HCl, HNO_3, $HClO_4$ and H_2SO_4 on the BDD electrode in the potential range from 0 to +2 V with a scan rate of 100 mV s^{-1}.

3.2 Effect of scan rate

A relevant parameter, which plays an important role in determining the nature of the electrode reaction of the analyte taking place on the working electrode, is the scan rate (ν). For this purpose, the effect of the scan rate on the current response of 1 mM SYN in 2 M $HClO_4$ was examined by evaluating several CV scans ranging from 5 to 300 mV s^{-1} on the BDD electrode. The CV curves show (Fig. 4) that with the increasing scan rate value, an increase of the peak current (I_p) of SYN was observed under the chosen experimental conditions. At the same time, there was a moderate displacement of the peak potential (E_p) of SYN to more positive values (approximately from +1.30 to +1.45 V), which is a characteristic feature of the irreversible behavior of the analyte.[34–37] Furthermore, a linear dependence of I_p against the square root of the scan rate ($\nu^{1/2}$) was observed (eqn (2)) within the studied range of the scan rate, suggesting that the electrochemical oxidation of SYN on the BDD electrode is unambiguously diffusion-driven. This phenomenon may also be confirmed by plotting $\log I_p = f(\log \nu)$ with a slope of 0.45 (eqn (3)), which is in good agreement with the theoretical value of 0.5 typical for diffusion-controlled reactions.[38]

$$I_p\ (\mu\mathrm{A}) = 2.82(\pm 0.57) + 1.05(\pm 0.06) \times \nu^{1/2},\ (R^2 = 0.9917) \quad (2)$$

$$\log I_p = -5.50(\pm 0.03) + 0.45(\pm 0.02) \times \log \nu,\ (R^2 = 0.9957) \quad (3)$$

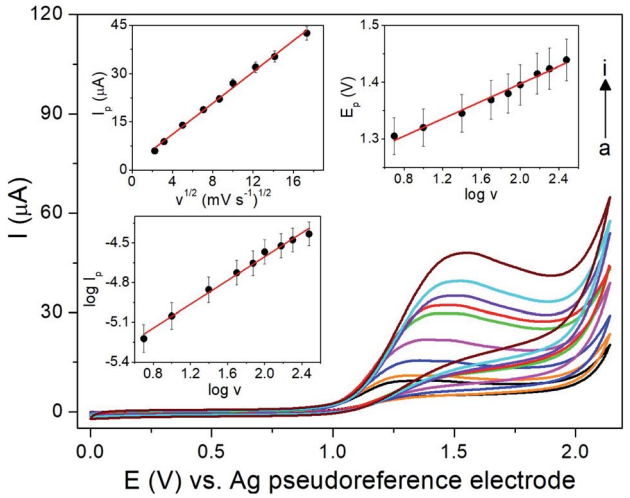
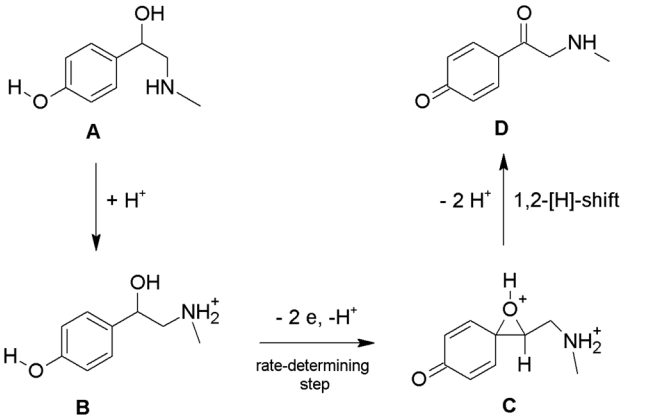


Fig. 4 CV profiles of 1 mM SYN in 2 M $HClO_4$ at different values of the scan rate: (a) 5, (b) 10, (c) 25, (d) 50, (e) 75, (f) 100, (g) 150, (h) 200 and (i) 300 mV s^{-1} on the BDD electrode. The dependencies $I_p = f(\nu^{1/2})$, log I_p = f(log ν) and $E_p = f$(log ν) are shown as an inset in the figure.



Scheme 1 The postulate of the oxidation mechanism of SYN on the BDD electrode.

Differently, in the case of previously published work dealing with the electrochemical behavior study of SYN on chemically modified electrodes,[22,23] the effect of scan rate confirmed adsorption to be the rate-determining step in the electrode reaction of SYN.

3.3 Postulate of the oxidation mechanism of SYN

Fully revealing the mechanism of electrochemical oxidation of SYN on the BDD electrode is not the main objective of this study. However, based on the results obtained under the selected experimental conditions, it is also desirable to postulate the oxidation mechanism of SYN on the BDD electrode. As given in Section 3.1, the slope of 0.028 V per pH for $E_p = f$(pH) revealed that the ratio between the number of exchanged electrons (z) and protons (n) is about 2.1. Afterwards, the number of electrons participating in the electrochemical oxidation of SYN was calculated according to the Laviron equation for irreversible electrochemical processes[38] as presented in the graph of $E_p = f$(log ν) in Fig. 4. After taking into consideration the values of the gas constant R (8.314 J K^{-1} mol^{-1}), temperature T (298 K), Faraday constant F (96 485 C mol^{-1}) and transfer coefficient α (its value for an irreversible system is 0.5), the particular equation (eqn (4)) can be expressed as:

$$E_p\,(\mathrm{V}) = 1.243(\pm 0.006) + 0.069(\pm 0.003) \times \log \nu,\ (R^2 = 0.9889) \quad (4)$$

According to the slope value ($2.3RT/\alpha zF = 0.069$), the number of exchanged electrons z was estimated to be 1.75 (*i.e.*, after approximation, 2), which means that the number of protons n was 1. This postulate is not in line with those stated on chemically modified electrodes (MWCNTs/Nafion/GCE[22] and MXene/MWCNTs/GCE[23]) where the authors outlined the participation of two electrons and two protons in one step during the electrode reaction of SYN. In general, electrooxidation of phenols is similar to that for aromatic primary amines and is highly dependent on the pH of the medium.[39] The explanation of our postulate could be as follows (Scheme 1): in an acidic environment (BR buffer with pH 3) SYN (labeled **A**) is first protonated. Subsequently, the oxidation of the particular phenol **B** (accompanied by two electron and one proton exchange as the rate-determining step), followed by intramolecular A_dN attack of hydroxy groups, may lead to intermediate spirobenzoquinone **C**. The spirobenzoquinone could fragment by 1,2-hydride rearrangement and final deprotonation provides the final product benzoquinone **D**.

3.4 Analytical performance evaluation and method verification

Pulse voltammetric techniques are increasingly being used in trace analysis because they are able to provide low LOD values (<1 μM) by the application of pulses, which significantly reduce the capacitive current. The choice of instrumental parameters of pulse techniques plays a significant role in trace determination from the viewpoint of method sensitivity and selectivity as well as the whole manifestation of voltammetric peaks of the particular analyte. Herein, in order to procure well-defined oxidation peaks with the highest magnitude and best peak shapes for further quantitative analysis, the instrumental parameters of DPV and SWV were required to be refined. For this purpose, 1 mM SYN in 2 M $HClO_4$ was used.

In the first step, suitable values of instrumental parameters for DPV were investigated. The measurements were performed by gradually changing the modulation time in the range from 10 to 200 ms, while the modulation amplitude was set to a constant value of 25 mV. Fig. 5 (inset) depicts a slight decrease of the background current as modulation amplitude increased. Applying the values of 50 and 75 ms, the recorded current responses of SYN were comparable. The highest current response (2.6 μA) with a sufficiently low background current was recorded at a modulation time of 100 ms, while after a subsequent increase to 150 and 200 ms, a decrease of the oxidation peak of SYN was just registered. Taking into account the highest achieved current response of SYN, 100 ms was chosen as the most suitable value. The search for an appropriate modulation

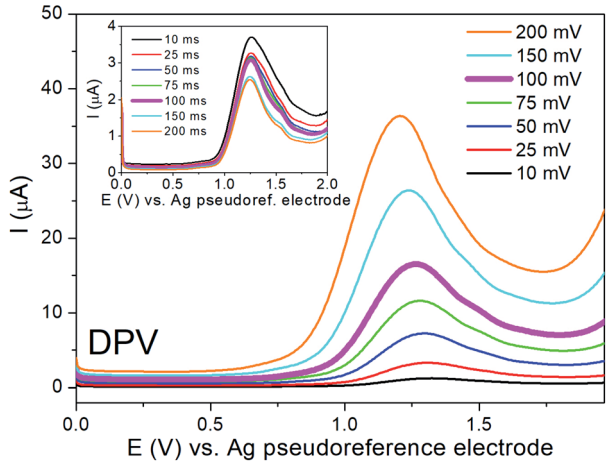


Fig. 5 DPV curves for 1 mM SYN in 2 M $HClO_4$ on the BDD electrode for different modulation amplitudes ranging from 10 to 200 mV at a fixed modulation time of 50 ms. Inset: DPV curves for 1 mM SYN in 2 M $HClO_4$ on the BDD electrode for different modulation times in the range of 10–200 ms at a fixed modulation amplitude of 25 mV.

amplitude value was performed in the same manner. Its values gradually varied in the range from 10 to 200 mV, while the modulation time was set to 50 ms. Evidently, a gradual increase of the modulation amplitude led to an increase in the oxidation peak of SYN in DP voltammograms. Besides, its slight shift towards more negative potential values was observed. At higher values of 150 and 200 mV, a mild increase of the background current was observed simultaneously with peak broadening. For this reason, 100 mV was chosen as the most appropriate value of the modulation amplitude.

With regard to SWV, at first glance (inset of Fig. 6) it is evident that the current response of SYN increased with the growing value of frequency, but at the same time there was a significant increase in background current observed within 50–150 Hz. As a frequency of 10 Hz provided an adequate current response of SYN not significantly affected by the background current, this value was evaluated to be the most favorable. When examining the suitable amplitude, the values of this parameter were gradually changed in the range of 10–150 mV, while frequency was constant (25 Hz). Accordingly, with the increasing value of amplitude, there was an increase in the oxidation peak of SYN recorded in SW voltammograms (Fig. 6). At a value of 150 mV, a significant increase in the background current was also shown. Due to the significant current response and the low current background value, 100 mV was selected as a reasonable value. The chosen values of instrumental parameters for DPV and SWV were subsequently used in all voltammetric analyses.

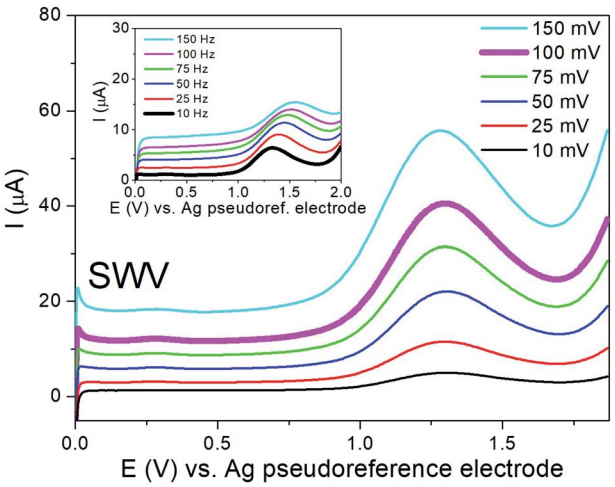


Fig. 6 SWV profiles for 1 mM SYN in 2 M $HClO_4$ on the BDD electrode for different amplitudes ranging from 10 to 150 mV at a fixed frequency of 25 Hz. Inset: SWV profiles for 1 mM SYN in 2 M $HClO_4$ on the BDD electrode for different frequencies in the range of 10–150 Hz at a fixed amplitude of 20 mV.

The quantification of SYN was undertaken under the selected experimental conditions using different SYN concentrations. The calibration curves were obtained by plotting the peak current (I_p) against the increasing SYN concentration (c_{SYN}). The basic analytical parameters such as sensitivity (slope of the calibration curve), linear concentration range, linearity, LOD and repeatability (precision) were estimated for both pulse techniques. They are summarized in Table 1, whereas the corresponding DP and SW voltammograms are presented in Fig. 7 and 8, respectively. The acquired sensitivities appeared to be almost similar (around 0.017 μA μM^{-1}) for both pulse techniques resulting in very close micromolar LOD values. Unquestionably, the data from the scientific literature have mentioned prospective electrochemical sensors based on chemically modified electrode surfaces[22–24] with higher sensitivity and lower LODs for SYN determination when compared to the BDD sensor presented herein. However, as also stated in the Introduction, there are some issues and obstacles connected with the preparation of chemically modified electrodes, especially from the viewpoint of procedure time, incorporation of a modifier onto the electrode surface, tedious synthesis of modifier material, *etc.* which could give rise to a substantially higher background and unrepeatable results.[25] Apart from this aspect, the BDD electrode within the presented voltammetric platform should serve as a simple and progressive electrochemical sensor for determination of SYN, exclusively only in dietary supplements with anticipated higher SYN amounts (mg). The intra-day repeatability of the developed method, which also characterized the stability of the presented voltammetric

Table 1 The analytical parameters for the determination of SYN on the BDD electrode using DPV and SWV

	Pulse voltammetric technique	
Parameter	DPV	SWV
Intercept (μA)	0.7682 ± 0.0573	1.1146 ± 0.0499
Slope (μA μM^{-1})	0.0166 ± 0.0004	0.0173 ± 0.0004
Linear concentration range (μM)	19.6–1000	9.9–1000
Linearity R^2	0.9957	0.9934
LOD (μM)	10.4	8.7
Repeatability[a] (RSD%, $n = 10$)	3.9	3.0

[a] For 19.6 μM SYN.

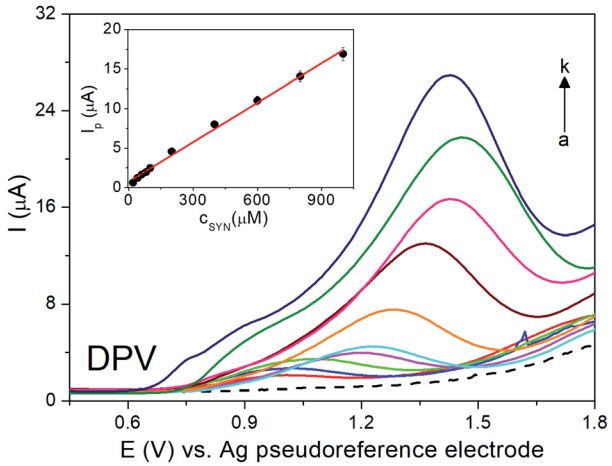


Fig. 7 DPV profiles for various SYN concentrations: (a) 0, (b) 19.6, (c) 40.0, (d) 60.0, (e) 80.0, (f) 100, (g) 200, (h) 400, (i) 600, (j) 800 and (k) 1000 μM in 2 M $HClO_4$ on the BDD electrode under the suitable DPV instrumental parameters. The calibration graph $I_p = f(c_{SYN})$ is shown as an inset in the figure.

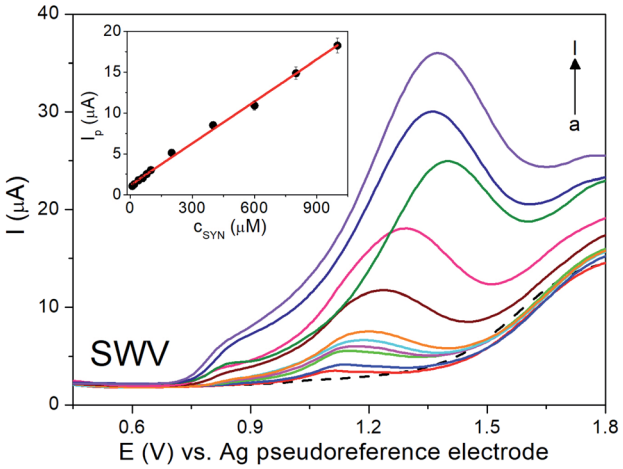


Fig. 8 SWV profiles for various SYN concentrations: (a) 0, (b) 9.90, (c) 19.6, (d) 40.0, (e) 60.0, (f) 80.0, (g) 100, (h) 200, (i) 400, (j) 600, (k) 800 and (l) 1000 μM in 2 M $HClO_4$ on the BDD electrode under the suitable SWV instrumental parameters. The calibration graph $I_p = f(c_{SYN})$ is shown as an inset in the figure.

platform, was explored for 10 replicate measurements for 19.6 μM SYN (first and second concentration level in the linear range for DPV and SWV, respectively) with the obtained relative standard deviation (RSD) lower than 4% for both pulse techniques. Based on the attained results it can be concluded that the BDD electrode provides adequate sensitivity with the repeatable current responses of SYN within the proposed platform which designate it for successful analysis of dietary supplements.

3.5 Interference study

In order to scrutinize the selectivity of the proposed voltammetric platform under the selected experimental conditions, increasing concentrations of the possible interfering agents, which can be typically found in food, were added to an electrochemical cell containing 40 μM SYN in 2 M $HClO_4$. Subsequently, the corresponding SW voltammograms were recorded and the effect was assessed through the ratio between the current response achieved in the analyzed solution containing the mixture of SYN and the particular interfering agent and the current response observed from a solution containing only SYN. The SWV technique was selected for the interference study because of its extreme rapidity and slightly higher sensitivity (lower LOD) when compared to the DPV technique for SYN determination. The tolerance limit was defined as the maximum concentration of a potential interfering substance that causes a change in the oxidation signal of ±10% for the determination of SYN. The results showed that a 200-fold excess of inorganic ions such as Na^+, Cl^-, NO_3^-, Ca^{2+}, Mg^{2+}, Cu^{2+}, and Zn^{2+} and a 100-fold excess of sugars (glucose, fructose, sucrose, and starch) as well as a 50-fold excess of ascorbic acid had almost no or negligible influence on the current responses of SYN (signal change lower than 5%). However, substantial interference may occur when the methylxanthine alkaloid caffeine (CAF) is present together with SYN in the analyzed solution. For demonstration, Fig. 9 displays the SW voltammograms demonstrating the influence of the presence of CAF on the current response of 40 mM SYN in 2 M $HClO_4$ on the BDD electrode. The peak-to-peak potential differences between SYN (+1.23 V) and CAF (+1.53 V) appeared to not be so far thus bringing about possible interference under certain circumstances. As can be seen, I_p and E_p of SYN were not significantly affected in the presence of CAF at a concentration ratio of 1 : 1 (signal change was about 10%). In the case of the 5-fold and 10-fold excess of CAF, a mild decrease of the SYN signal, caused by its overlapping with the oxidation peak of CAF, was observed. However, the selectivity became worse when taking into account the 25-fold and 50-fold excess of CAF since there was a remarkable overlapping of the oxidation peaks of SYN and CAF recorded in SW voltammograms. In these cases, the peak current of SYN decreased radically (more than 50%) and this

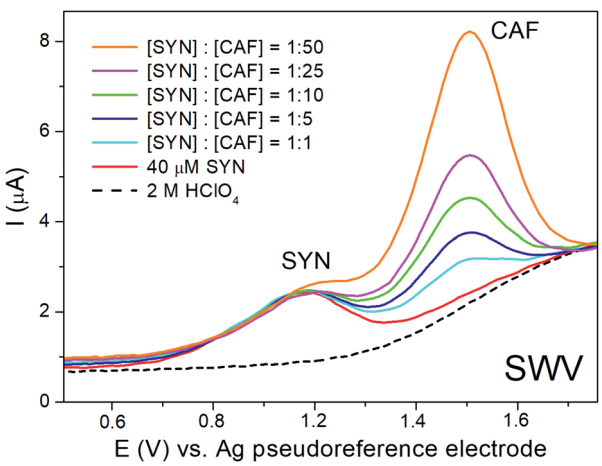


Fig. 9 SWV profiles demonstrating the influence of the presence of caffeine (CAF) on the current response of 40 μM SYN in 2 M $HClO_4$ on the BDD electrode.

Table 2 Analysis of commercially available SYN-containing dietary supplements using DPV and SWV on the BDD electrode under the selected experimental conditions ($n = 3$)

		Determined amount (mg)		Recovery (%)	
Dietary supplement	Label (mg)	DPV	SWV	DPV	SWV
Syneslim	5	4.8 ± 0.4	5.1 ± 0.6	96	102
Synephrine	10	9.8 ± 0.9	10.2 ± 1.0	98	102
Thermo virus	10	9.7 ± 1.1	10.1 ± 1.3	97	101
Warrior fat burner	15	14.8 ± 0.5	14.4 ± 0.8	99	96

phenomenon was accompanied by a slight E_p shift of SYN towards more positive values. To sum up, the utilization of the developed method in sample analysis could be partially restricted by the presence of higher amounts of CAF giving rise to lower selectivity. However, at the same time, admitting the fact that all dietary supplements analyzed in this work are CAF-free, it should be stated that the selectivity of the proposed voltammetric platform could be considered adequate.

3.6 Analysis of dietary supplements

The viability of the proposed voltammetric platform was investigated by the determination of SYN in four commercially available dietary supplements with the different declared amounts of SYN (from 5 to 15 mg per dosage) depending on the brand. The detailed information about the sample preparation and electrochemical analysis is mentioned in Section 2.4. Table 2 shows the determined amounts of SYN in the dietary supplements achieved by the DPV and SWV procedures on the BDD electrode under the selected experimental conditions using the multiple standard addition method. The average results for three successive DPV and SWV measurements are expressed as a confidence interval for 95% probability. Evidently, the results are in good agreement with the labeled SYN contents in the samples and the determined recovery values ranged from 96 to 102% thus verifying the comfortable accuracy of the developed voltammetric platform and revealing its feasibility for food quality control analysis. The Illustrative example of the analysis of the commercial dietary supplement Synephrine GymBeam using SWV is depicted in Fig. 10. The graphical evaluation of the multiple standard addition method is given as an inset.

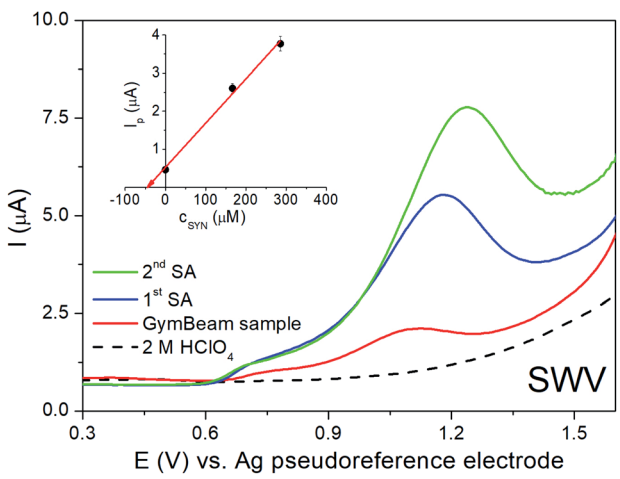


Fig. 10 SWV profiles for the determination of SYN in the commercially available dietary supplement Synephrine GymBeam in 2 M $HClO_4$ on the BDD electrode using the multiple standard addition method under the suitable experimental conditions. The graphical evaluation of the multiple standard addition (SA) method is shown as an inset in the figure.

4. Conclusions

Despite the presence of the oxidizing phenolic hydroxyl group in the molecular structure of SYN, up to now the scientific literature has reflected only a few reports on electrochemical study and determination of this compound. For this purpose, the primary attention of most analysts and electrochemists has been recently focused on the utilization of chemically modified electrodes owing to the excellent physical and chemical properties. In this work, for the first time, we propose a novel voltammetric platform based on the usage of the commercial screen-printed BDD electrode as a progressive electrochemical sensor for simple, fast and reliable quantification of SYN. Under the selected experimental conditions, the novel voltammetric platform was developed using pulse techniques with the basic analytical parameters including LOD at micromolar concentration levels, wide linear concentration range and good repeatability. The interference study revealed that the method selectivity was found to be fair. The feasibility of the proposed voltammetric platform was successfully verified in the analysis of four commercially available dietary supplements. On the basis of the achieved results it can be concluded that the advanced electrochemical sensor based on the screen-printed BDD electrode is a suitable and unique competitor for efficient applications in food quality control analysis. In addition, the developed platform can be applied directly without higher consumption of chemicals, waste generation, complex sample extraction and higher instrumentation cost. Finally, this paper signifies the very first modification-free approach on the electrochemical behavior study and determination of SYN on the screen-printed BDD electrode.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the Grant Agency of the Slovak Republic VEGA (grant no. 1/0159/20), STU Grant Scheme for Support of Young Researchers and Grant scheme for Support of Excellent Teams of Young Researchers. DMS wishes to acknowledge the Ministry of Education, Science and Technological Development of the Republic of Serbia through Eureka project E! 13303 MED-BIO-TEST.

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